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Original article

Vitamin B12 deficiency and hyperhomocysteinemia as correlates of cardiovascular risk factors in Indian subjects with coronary artery disease

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ABSTRACT

Background and purpose: Folate and vitamin B12 are essential components in the metabolism of homocysteine (Hcy). Hyperhomocysteinemia has been implicated in endothelial dysfunction and cardiovascular disease. However, the association of Hcy, vitamin B12, and folic acid with cardiovascular risk factors in patients with coronary artery disease (CAD) has not been studied in Indian patients. This study was conducted with the aim to evaluate the relationship of vitamin B12, folic acid, and Hcy levels with cardiovascular risk factors in subjects with known CAD.
Methods and subjects: Three hundred patients (216 men; 84 women; aged 25–92 years) who had CAD on angiography were included in this study consecutively. All patients were evaluated for anthropometry and cardiovascular risk factors, and blood samples were collected for biochemical, nutritional, and inflammatory markers.
Results: Percentage of vitamin B12 and folate deficiency was 86.7% and 2.7%, respectively. Hyperhomocysteinemia was present in 95.3% patients. Vitamin B12 levels were significantly lower and Hcy levels were significantly higher in subjects with dyslipidemia, DM, and/or hypertension. Serum vitamin B12 was inversely associated with triglyceride and very low-density lipoprotein (VLDL) and positively with high-density lipoprotein (HDL). Hcy was positively associated with triglyceride and VLDL and negatively with HDL. Vitamin B12 was inversely correlated with inflammatory markers (high-sensitivity C-reactive protein and interleukin-6) directly related to insulin resistance whereas Hcy showed the opposite pattern.
Conclusions: Serum vitamin B12 deficiency and hyperhomocysteinemia are related with cardiovascular risk factors in Indian patients with CAD.

Introduction

Homocysteine (Hcy) is a sulfhydryl containing amino acid produced by demethylation of an essential amino acid (methionine). Methylation of Hcy, catalyzed by methionine synthetase produces methionine [1]. Folate and vitamin B12 are essential components in the metabolism of Hcy, which occurs through remethylation to methionine or trans-sulfuration to cysteine. The enzyme methylene-tetrahydro-folate-reductase (MTHFR) is responsible for the reduction of 5,10-methylene-THF to 5-methyl-THF, where vitamin B12 acts as a cofactor [2]. Hcy-mediated enhanced lipid peroxidation and generation of free radicals results in inflammation and acute endothelial dysfunction, which accelerates atherosclerotic process predisposing to cardiovascular disease. The first clinical study by Wilcken and Wilcken in 1976, supported the theory that coronary artery disease (CAD) is associated with higher levels of Hcy [3]. It has also been demonstrated that in the presence of traditional risk factors, Hcy plays a permissive role in endothelial damage. Low vitamin B12 concentration and hyperhomocysteinemia are common in Indian men, particularly in vegetarians and urban residents [4].

Many studies have been undertaken to examine the relation between plasma Hcy and coronary heart disease [5,6]. The general outcome supports the hypothesis that an elevated plasma Hcy concentration leads to an increased risk of cardiovascular disease. However, there are few studies that showed the association of Hcy, vitamin B12, and folic acid with cardiovascular risk factors in patients with known CAD. This study was conducted with the aim to evaluate the relationship of vitamin B12, folic acid, and Hcy levels with cardiovascular risk factors in subjects with known CAD.

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Materials and methods

Patients reporting with chest pain were evaluated by a cardiologist and underwent coronary angiography based on clinical and investigational data. Blood samples were collected and if CAD was detected on angiography, patients were included in the study. Patients with chronic kidney disease, hepatic dysfunction, known endocrinal (except diabetes mellitus) or rheumatologic diseases or chronic infections, and patients being treated with vitamins were excluded from the study. All cases were interviewed using a questionnaire, which included data on smoking and physical activity.

Height, weight, waist and hip circumference were measured. Body mass index (BMI) was calculated by dividing weight in kilograms with square of height in meters. Waist hip ratio (WHR) is waist circumference divided by hip circumference. Data on clinical history of hypertension (HTN) and diabetes mellitus (DM) and medications (antihypertensive, lipid lowering, and oral hypoglycemic agents) were also acquired. Conventional risk factors were defined as follows: BMI < 25 normal, ≥25 overweight/obese, DM (by history and treatment), and HTN (systolic and diastolic blood pressures above 140 and 90 mmHg, respectively).

Fasting blood samples were collected after 14 h of fasting. Lipids were measured by using CHOD PAP, LIP/GK, enzymatic reaction respectively and low-density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) were calculated by Freidewald formula. Inter assay 3.84% and intra precision was 2%, respectively for all biochemical parameters. Hemoglobin (Hb)A1C was measured by boronate affinity assay. Tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL6), highly sensitive C-reactive protein (hsCRP), and Hcy were measured by enzyme linked immunosorbent assay method with kits manufactured by Genprobe Diaclone, Besançon, France, Biocheck, Foster City, CA, USA, and Axis-shield Diagnostic Ltd., Dundee, UK respectively. Insulin, vitamin B12, and folic acid were measured by Microparticle enzyme immunoassay (MEIA), and Ion Capture MEIA method respectively with commercial kits supplied by Abbott Laboratory, Abbott Park, IL, USA. Intra assay and inter assay precision were <5% and <10%, respectively for above parameters. Insulin resistance and sensitivity was calculated by using homeostatic model analysis (HOMA) [HOMA-insulin resistance = fasting insulin (μIU/ml) × fasting glucose (mmol/l)/22.5 and quantitative insulin sensitivity check index (QUICKI)] [QUICKI = 1/log(fasting insulin μU/mL) + log(fasting glucose mg/dL)] respectively. Atherogenic dyslipidemia was defined as triglyceride level ≥ 1.69 mmol/L (≥150 mg/dl), and high-density lipoprotein cholesterol (HDL) cholesterol level < 1.03 mmol/L (<40 mg/dl). Vitamin B12 deficiency was defined by <147.6 pmol/L (<200 pg/ml), folate deficiency by <3 ng/ml, and hyperhomocysteinemia by >15 μmol/L (>2.02 mg/L). For comparison we have divided all subjects into three groups according to vitamin B12 levels: <73.8 pmol/L (<100 pg/ml) – group1; 73.8–147.6 pmol/L (100–200 pg/ml) – group 2; >147.6 pmol/L (>200 pg/ml) – group 3; and two groups according to Hcy levels: ≤15 μmol/l (≤2.02 mg/L) – group 1; >15 μmol/l (>2.02 mg/L) – group 2.

Informed written consent was obtained from all patients and the study protocol was approved by the institutional ethics and review committee.

Statistical analysis was carried out using EPI Info, version 3.5.3 (CDC; Atlanta, GA, USA) and SPSS Version 20 (Chicago, IL, USA). Data are presented as mean ± SD, median (range) or number (%) unless specified. All parametric data were analyzed by Student's *t*-test. If Bartlett's chi-square test for equality of population variances was <0.05 then Kruskal–Wallis test was applied. Pearson correlation was used to evaluate the correlation between inflammatory markers, insulin resistance, and nutritional factors. Multiple regression analysis was performed after adjustment for age, sex, BMI, and presence of HTN. All non parametric data were analyzed by chi-square test. A *p*-value of <0.05 was considered statistically significant.

Results

Three hundred patients with known cardiovascular disease (216 men; 84 women; aged 25–92 years) were studied. The percentages with vitamin B12 and folate deficiency were 86.7% and 2.7%, respectively. Subjects with hyperhomocysteinemia were 95.3%. Table 1 shows the baseline characteristics of the subjects studied. There was no gender difference in vitamin B12 and folic acid (Table 1).

Table 1
Basic characteristics of the study population.

Parameters	Male (*n* = 216) Mean ± SD	Female (*n* = 84) Mean ± SD	*p*-Value
Age (years)	60.8 ± 12.3	61.03 ± 12.9	0.9205
BMI (kg/m^2)	27.65 ± 3.69	28.5 ± 4.07	0.0762
WHR	0.92 ± 0.05	0.91 ± 0.06	0.1185
DM, *n* (%)	86(68.8%)	39 (31.2%)	0.3613
HTN, *n* (%)	72 (75.8%)	23 (24.2%)	0.3914
DM & HTN, *n* (%)	59 (63.4%)	34 (36.6%)	0.038
Smoking, *n* (%)	82 (73.9%)	29 (26.1%)	0.6738
Dyslipidemia, *n* (%)	81 (65.3%)	43 (34.7%)	0.0422
Cholesterol, mmol/l (mg/dl)	4.5 ± 1.1 (177.7 ± 44.4)	4.6 ± 1.2 (181.2 ± 48.8)	0.5505
Triglyceride, mmol/l (mg/dl)	2.24 ± 0.51 (198.8 ± 45.4)	1.97 ± 0.54 (174.5 ± 48.3)	0.428
HDL, mmol/l (mg/dl)	1.0 ± 0.23 (39.3 ± 9.2)	0.9 ± 0.23 (38 ± 9.2)	0.2528
LDL, mmol/L (mg/dl)	2.7 ± 1.27 (104.4 ± 49.2)	2.8 ± 1.39 (108.3 ± 53.8)	0.5468
VLDL, mmol/L (mg/dl)	0.86 ± 0.24 (33.4 ± 9.6)	0.88 ± 0.27 (34.1 ± 10.7)	0.6378
Insulin, pmol/l (mU/L)	342.3 ± 288.2 (49.3 ± 41.5)	362.5 ± 334.7 (52.2 ± 48.2)	0.6018
HOMA IR	16.6 ± 17.01	22.2 ± 28.6	0.5029
QUICKI	0.28 ± 0.039	0.27 ± 0.047	0.4649
IL-6 (pg/ml)	62.3 ± 73.8	70.1 ± 78.9	0.422
TNF-α (pg/ml)	22.8 ± 42.2	28 ± 38.7	0.3373
hsCRP (mg/L)	11.9 ± 10.03	11.1 ± 8.72	0.556
Vitamin B12, pmol/L (pg/ml)	167.4 ± 297.7 (226.9 ± 403.5)	99.7 ± 64.5 (135.2 ± 87.5)	0.0738
Homocysteine, μmol/L (mg/L)	36.5 ± 13.58 (4.93 ± 1.83)	35.0 ± 18.77 (4.73 ± 2.53)	0.0269
Folic acid, nmol/L (ng/ml)	18.1 ± 16 (8.0 ± 7.1)	17.6 ± 15.1 (7.8 ± 6.7)	0.7980

BMI, body mass index; WHR, waist hip ratio; DM, diabetes mellitus; HTN, hypertension; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; VLDL, very low density lipoprotein cholesterol; HOMA IR, homeostatic model analysis calculated insulin resistance; QUICKI, quantitative insulin sensitivity check index; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; hsCRP, highly sensitive C-reactive protein.

Subjects with DM and/or HTN had significantly lower vitamin B12 levels (Table 2). Serum vitamin B12 concentration was inversely related with inflammatory markers and was positively related to insulin sensitivity (Table 3). Vitamin B12 levels were low in subjects with dyslipidemia (Table 2). Serum vitamin B12 was negatively correlated with dyslipidemia (Table 3) and this association remained unchanged even after adjustment for all other risk factors: age, sex, BMI, WHR, physical inactivity, smoking, and diabetes (Supplementary Table 1). Serum vitamin B12 was inversely associated with triglycerides and VLDL, and positively with HDL after adjustment with other risk factors. However, there was no association of vitamin B12 with total cholesterol and LDL cholesterol (Table 4).

Subjects with DM and/or HTN had significantly higher Hcy levels (Table 2). Subjects with dyslipidemia had significantly higher Hcy levels. Hcy was positively correlated with dyslipidemia (Table 2) which persisted in stepwise multiple regression analysis even after adjustment with other risk factors: sex, BMI, WHR, physical inactivity, smoking, and diabetes (Supplementary Table 2). Hcy was positively correlated with insulin, insulin resistance, IL-6, and hsCRP (Table 3 and Fig. 1). Hcy was positively associated with triglyceride and VLDL, and negatively with HDL after adjustment with all risk factors (Table 4 and Fig. 1). However, there was no association of Hcy with total cholesterol and LDL cholesterol (Table 4).

All subjects were grouped according to vitamin B12 levels and Hcy levels. Insulin levels and HOMA-IR decreased from group 1 to group 3; whereas insulin secretion assessed by QUICKI improved. Similarly inflammatory markers (hsCRP, IL-6, and TNF-α) also decreased from group 1 to group 3. Subjects with

Table 2
Vitamin B12, homocysteine, and folic acid levels according to cardiovascular risk factors.

	Vitamin B12 pmol/L (pg/ml)		Homocysteine\ μmol/L (mg/L)		Folic acid nmol/L (ng/ml)	
	Mean ± SD	*p*-Value	Mean ± SD	*p*-Value	Mean ± SD	*p*-Value
DM						
Yes	131.5 ± 222.2 (178.2 ± 301.2)	0.0014	39.2 ± 15.9 (5.29 ± 2.14)	0.0026	17.4 ± 14.2 (7.7 ± 6.3)	0.5116
No	160.2 ± 278.5 (217.2 ± 377.4)		33.9 ± 14.2 (4.58 ± 1.91)		18.5 ± 16.7 (8.2 ± 7.4)	
Smoking						
Yes	148 ± 245.3 (200.6 ± 332.4)	0.9893	36.0 ± 13.2 (4.86 ± 1.78)	0.5104	17.9 ± 15.4 (7.9 ± 6.8)	0.9517
No	148.4 ± 263.6 (201.2 ± 357.3)		36.1 ± 16.2 (4.88 ± 2.19)		18.1 ± 16 (8.0 ± 7.1)	
HTN						
Yes	139.9 ± 259.7 (189.6 ± 352.0)	0.4639	38.1 ± 15.8 (5.15 ± 2.13)	0.0036	16.9 ± 14.5 (7.5 ± 6.4)	0.2252
No	162.4 ± 152.6 (220.1 ± 341.0)		32.8 ± 13.5 (4.43 ± 1.82)		19.9 ± 17.9 (8.8 ± 7.9)	
DM & HTN						
Yes	119.3 ± 202.5 (161.7 ± 274.5)	0.0002	40.3 ± 16.0 (5.44 ± 2.16)	0.0011	16.9 ± 13.5 (7.5 ± 6.0)	0.4439
No	161.5 ± 276.9 (218.9 ± 375.3)		34.2 ± 14.4 (4.62 ± 1.94)		18.5 ± 16.7 (8.2 ± 7.4)	
Dyslipidemia						
Yes	64.8 ± 6.2 (87.9 ± 8.5)	<0.0001	50.0 ± 12.4 (6.75 ± 1.67)	<0.0001	19.7 ± 16.9 (8.7 ± 7.5)	0.1381
No	207 ± 322.5 (280.6 ± 437.1)		26.3 ± 7.2 (3.55 ± 0.97)		16.9 ± 14.7 (7.5 ± 6.5)	
Physical inactivity						
Yes	129 ± 177.3 (174.9 ± 240.3)	0.5423	36.2 ± 15.7 (4.89 ± 2.12)	0.9267	16.3 ± 14 (7.2 ± 6.2)	0.1617
No	160.4 ± 295.7 (217.4 ± 400.7)		36.1 ± 14.9 (4.88 ± 2.01)		19 ± 16.7 (8.4 ± 7.4)	
BMI						
Normal < 25	126.7 ± 142.3 (171.8 ± 192.9)	0.4456	36.8 ± 17.9 (4.97 ± 2.41)	0.8742	18.8 ± 16.7 (8.3 ± 7.4)	0.6713
Overweight/obese ≥ 25	154.2 ± 280 (209.0 ± 379.5)		35.9 ± 14.3 (4.85 ± 1.93)		17.9 ± 15.6 (7.9 ± 6.9)	
WHR						
≤0.9	178.2 ± 323.4 (241.5 ± 438.3)	0.5249	36.2 ± 15.4 (4.89 ± 2.08)	0.9317	17.6 ± 15.1 (7.8 ± 6.7)	0.8032
>0.9	135.4 ± 221.5 (183.6 ± 300.2)		36.0 ± 15.1 (4.86 ± 2.04)		18.1 ± 16 (8.0 ± 7.1)	

DM, diabetes mellitus; HTN, hypertension; BMI, body mass index; WHR, waist hip ratio.

Table 3
Correlation of vitamin B12, homocysteine, and folic acid with cardiovascular risk factors.

	Vitamin B12		Homocysteine		Folic acid	
	r-Value	*p*-Value	*r*-Value	*p*-Value	*r*-Value	*p*-Value
Insulin	−0.102	0.077	0.313	<0.0001	−0.058	0.315
HOMA IR	−0.124	0.032	0.377	<0.0001	−0.017	0.775
QUICKI	0.105	0.070	−0.365	<0.0001	−0.093	0.106
IL-6	−0.091	0.116	0.296	<0.0001	0.050	0.387
hsCRP	−0.102	0.078	0.313	<0.0001	0.023	0.685
TNF alpha	−0.016	0.784	0.099	0.088	−0.028	0.627
DM	−0.055	0.339	0.173	0.003	−0.027	0.636
Smoking	−0.001	0.989	−0.003	0.965	−0.019	0.741
HTN	−0.042	0.464	0.168	0.004	−0.092	0.111
DM & HTN	−0.076	0.190	0.187	0.001	−0.042	0.471
Dyslipidemia	−0.273	<0.0001	0.770	<0.0001	0.068	0.241
Physical inactivity	−0.060	0.302	0.007	0.898	−0.041	0.482
BMI	0.028	0.634	−0.041	0.474	0.043	0.459
WHR	−0.026	0.659	−0.022	0.704	−0.001	0.987

HOMA IR, homeostatic model analysis calculated insulin resistance; QUICKI, quantitative insulin sensitivity check index; IL-6, interleukin 6; hsCRP, highly sensitive C-reactive protein; TNF, tumor necrosis factor; DM, diabetes mellitus; HTN, hypertension; BMI, body mass index; WHR, waist hip ratio.

hyperhomocysteinemia were more insulin resistant (HOMA-IR) and had decreased beta cell function (QUICKI). hsCRP levels were significantly higher in subjects with hyperhomocysteinemia (Supplementary Table 3). There was no correlation between body mass index, WHR, and physical activity with folic acid. Folic acid levels did not differ and show any relation with any risk factors. Folic acid levels were comparable in subjects with and without dyslipidemia.

Discussion

Increased plasma Hcy levels are positively associated with new onset CAD, recurrent cardiovascular events, extent of myocardial damage, and mortality in patients with ischemic heart disease [7,8]. Elevated homocysteine levels are also related to intima media thickness [9] and to severity of CAD in Japanese patients [10], but were unrelated to major cardiovascular adverse events in patients with CAD who had undergone percutaneous coronary intervention [11]. However, few studies with small numbers of subjects were able to demonstrate this relation [1,12]. It has been postulated that higher Hcy levels lead to epigenetic modification by macromolecular global DNA methylation, which is associated with CAD in Indian patients [13]. We evaluated the association of Hcy levels and related nutritional markers (vitamin B12, folic acid) with known cardiovascular risk factors in 300 subjects with CAD. Most of the patients were vitamin B12 deficient (86.7%) but a few were folate deficient (2.7%). Another study from India also reported high prevalence (87%) of vitamin B12 deficiency Vitamin B12 deficiency varied from 1.5% to 26.6% in other countries [2,14–16]. The differences in prevalence of serum vitamin B12 deficiency and folate can be explained on the basis of the different cutoff points defined for vitamin B12 deficiency and dietary intake of vitamin B12 according to dietary differences [17]. Hyperhomocysteinemia was present in 95.3% patients in the present study. High prevalence of hyperhomocysteinema was also reported from the same region of India in one study (79% in urban population) [18] and from northern part of India (84%) [17]. A lower prevalence of hyperhomocysteinemia

Table 4
Association of vitamin B12 and homocysteine with lipids.

	Univariate analysis with vitamin B12		Multiple regression with vitamin B12 (adjusted with all other risk factors)		Univariate analysis with homocysteine		Multiple regression with homocysteine (after adjustment with all other risk factors)	
	r-Value	*p*-Value	Beta coefficient	*p*-Value	*r*-Value	*p*-Value	Beta coefficient	*p*-Value
Cholesterol	−0.010	0.855	−0.032	0.9420	−0.026	0.651	−0.012	0.5306
Triglyceride	−0.140	0.015	−0.965	0.031	0.434	<0.0001	0.133	<0.0001
HDL	0.136	0.019	5.017	0.030	−0.442	<0.0001	−0.707	<0.0001
LDL	−0.009	0.882	−0.022	0.9570	−0.023	0.696	−0.010	0.5621
VLDL	−5.271	0.015	−4.825	0.031	0.434	<0.0001	0.665	<0.0001

HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

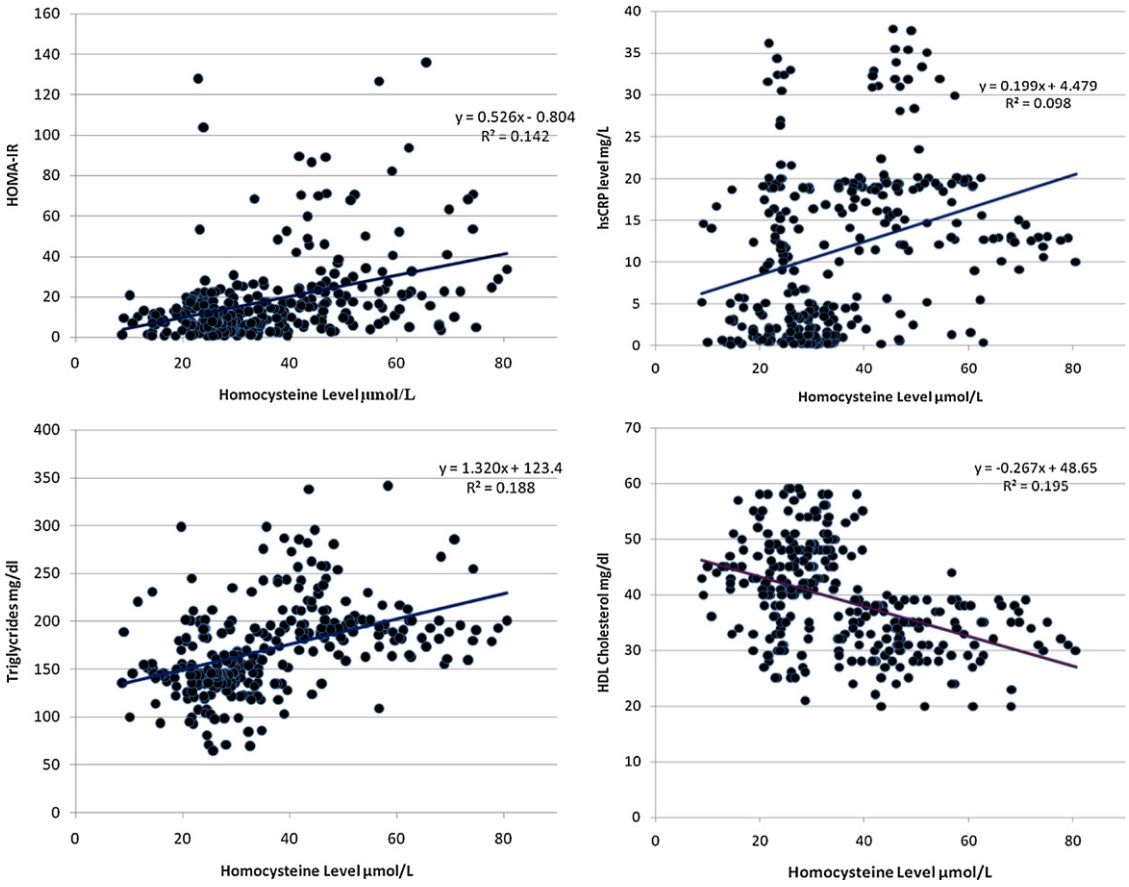

Fig. 1. Scatter plot with trendline showing relationship of homocysteine with HOMA-IR, hsCRP, triglycerides, and HDL levels. HOMA IR, homeostatic model analysis calculated insulin resistance; hsCRP, highly sensitive C-reactive protein; HDL, high-density lipoprotein cholesterol.

(6.1–47.4%) was reported from other countries [2,11,19,20]. Moreover, mean Hcy levels show a geographical pattern with lower levels reported from European countries [5–9], which increases on moving to Asian countries [1,2,16,17]. It has been suggested that inadequate plasma concentration of vitamin B12 is a contributing factor in approximately 2/3 of all cases of hyperhomocysteinemia [19] and low vitamin B12 concentration contributed 28% to the risk of hyperhomocysteinemia [4]. In this study we have observed higher mean Hcy levels because we have selected patients who already had CAD. The association of hyperhomocysteinemia with CAD seems stronger in Indian patients. In a case–control study in Indian subjects with or without CAD, age adjusted odds ratio for homocysteine (10.54, 95%CI 3.11–35.78) was second to smoking among various cardiovascular risk factors [21]. Hyperhomocysteinemia has been reported in Indian children and adolescents and found to be related to atherogenic dyslipidemia [22]. Higher levels of homocysteine have been observed in Indian subjects compared to migrant Indians [23]. Hence, homocysteine levels are an important determinant of cardiovascular risk factors in Indian subjects with or without CAD.

Serum vitamin B12 was higher in males as compared to females but was statistically comparable. A study from Korea among middle-income groups reported significantly lower levels in males than females [11]. Hcy levels were not correlated with age in the present study, but other studies have shown a positive correlation [11,19,20]. Hcy levels were higher in males compared to females in our study which has been also observed in other studies [7,24]. Similar to our study no correlation was detected among Hcy and BMI and WHR by others [25,26] whereas one study reported higher BMI and WHR in the group with high Hcy as compared to a group with normal Hcy levels [27]. There was no difference in levels of Hcy, vitamin B12, and folate levels between smokers and non-smokers. Others have reported lower levels of serum folate and higher Hcy in smokers when compared with non-smokers [28].

In the present study, vitamin B12 was lower in patients with DM and dyslipidemia but was independently associated with dyslipidemia only. Similar to our study, low serum levels of vitamin B12 were observed in Omani adults with DM [29]. Hcy was higher in DM and/or HTN subjects in the present study. Others have also observed higher Hcy levels in patients with DM [25,30]. One study reported higher blood pressure in high Hcy group as compared to the group with normal Hcy level [25].

Hcy levels were higher in subjects with lipid abnormalities than without them in our study. An association between hyperlipidemia and hyperhomocysteinemia has been reported by Obeid et al. [31]. Higher plasma Hcy was associated with lower HDL levels and higher triglyceride levels in our study. A similar association has been reported in Indian adolescents [22]. Hcy was unrelated to serum triglyceride and HDL cholesterol levels in one study [25]. Hcy accumulation leads to the synthesis and accumulation of *S*-adenosyl-L-homocysteine, which is an inhibitor of *S*-adenosyl-L-methionine-dependent methyltransferases and responsible for methylation of nucleic acids, proteins, and lipids. This will cause hypomethylation of various enzymes and accumulation of lipids in liver along with increased levels of triacyglycerols [32]. Decreased methyl group will decrease the synthesis of phosphatidylcholine, major phospholipids required for VLDL assembly and homeostasis [31].

Vitamin B12 levels were negatively correlated with HOMA-IR and insulin levels in the present study. A similar association has been observed in women with polycystic ovary syndrome [33]. Contrary to this, a cross-sectional study conducted in 135 Asian Indian women found no correlation between serum vitamin B12 and HOMA IR [16]. Vitamin B12 treatment improved insulin resistance and endothelial dysfunction, along with decreasing Hcy levels, in patients with metabolic syndrome, suggesting that vitamin B12 has several beneficial effects on cardiovascular disease risk factors. However, a systematic review of cohort studies showed limited evidence of vitamin B12 deficiency and morbidity and mortality from cardiovascular disease [34], and a meta-analysis of several studies done with vitamin B12 and folate supplementation failed to show a decrease in coronary artery events [35].

In the present study, subjects with hyperhomocysteinemia had significantly high HOMA IR index but insulin levels were comparable. Hcy had a positive association with both insulin and HOMA IR. The Framingham offspring study demonstrated a modest association between hyperinsulinemia and fasting Hcy levels [36]. In Japanese diabetic patients, insulin resistance was an independent predictor of total Hcy levels and insulin and HOMA IR were higher in high Hcy group in subjects with type 2 DM as compared to a group with normal Hcy levels [26]. However, other studies failed to show a correlation between Hcy and insulin sensitivity in male patients [25] and in healthy pre-menopausal South Asian women [37].

In our study, Hcy levels were correlated with IL-6 and hsCRP levels. A large observational study among women (Nurses' Health Study) found a positive association between total Hcy and cytokines IL-6 and CRP along with soluble TNF receptor. A large number of studies have provided evidence of the role of inflammation in the development and progression of atherosclerotic processes [16,33]. This may in part explain the observed association between high circulating concentrations of Hcy and cardiovascular diseases described in many observational studies [1–3].

In the present study, Hcy was inversely associated with plasma vitamin B12 ($r = -0.285$, $p < 0.001$). Hcy exhibited an inverse association with plasma folate ($r = -0.3$ to -0.37) and vitamin B12 ($r = -0.2$ to -0.22) in other studies [1,14,19,20]. Contrary to the most of the studies, we were unable to observe an inverse relation between serum folate levels. In a prospective study using Mendelian randomization, it was shown that individuals with MTHFR 677TT genotype had elevated Hcy with low-normal folate, whereas those with high-normal folate had normal Hcy concentrations [38]. A similar observation was made by Indian studies [39]. A study in an Indian population revealed a significant association of Hcy levels with MTHFR A1298C polymorphism which was more common than MTHFR 677TT genotype [40]. However, other studies were unable to demonstrate a relation between Hcy levels and MTHFR 677TT polymorphism [41]. This may explain the observed association of folate with Hcy in western populations. Folate acts indirectly through vitamin B12 as a methyl donor to Hcy. Hence, a correlation of serum folate and Hcy is evident in studies in populations with vitamin B12 sufficiency. In our population, there was high intake of folic acid and low percentage of folic acid deficiency in the presence of high vitamin B12 deficiency, which may have masked the relation between serum folate and Hcy [39].

There were some limitations to our study. Firstly, we have not taken a control group. We wanted to study a population with confirmed CAD because they are more likely to reveal alterations in inflammatory markers, insulin resistance, and dietary factors being at the extreme end of the disease spectrum. This will help us in revealing correlations among these factors. Moreover, apparently healthy asymptomatic controls do not necessarily have absence of underlying subclinical CAD. Secondly, being a cross-sectional study, long-term follow-up data were not available. Thirdly, we have not included dietary data, as they are part of another paper under review.

Conclusion

Vitamin B12 deficiency and hyperhomocysteinemia were associated with traditional and non-traditional cardiovascular risk

factors and were independently associated with dyslipidemia even after adjustment for all other risk factors in Indian patients with CAD. Thus, vitamin B12 deficiency causing hyperhomocysteinemia may be a risk factor for cardiovascular disease and important for prediction of future cardiovascular disease.

Conflict of interest

None of the authors have any conflicts of interests.

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Nothing to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jjcc.2012.11.009.

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